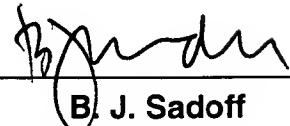


MAERTENS et al.
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Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____



B. J. Sadoff
Reg. No. 36,663

BJS:eaw

1100 North Glebe Road, 8th Floor
Arlington, VA 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

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Example 1: Cloning and expression of the hepatitis C virus E1 protein

1. Construction of vaccinia virus recombination vectors

The pgptATA18 vaccinia recombination plasmid is a modified version of pATA18 (Stunnenberg et al, 1988) with an additional insertion containing the *E. coli* xanthine guanine phosphoribosyl transferase gene under the control of the vaccinia virus I3 intermediate promoter (Figure 1). The plasmid pgsATA18 was constructed by inserting an oligonucleotide linker with SEQ ID NO 1/94, containing stop codons in the three reading frames, into the Pst I and HindIII-cut pATA18 vector. This created an extra Pac I restriction site (Figure 2). The original HindIII site was not restored.

Oligonucleotide linker with SEQ ID NO 1/94:

5'	G GCATGC AAGCTT AATTAAATT	3'
3'	ACGTC CGTAGC TTCAAA TTAATTAA TCGA	5'
<hr style="display: inline-block; width: 150px; border: none; border-top: 1px solid black; margin-right: 10px;"/> <hr style="display: inline-block; width: 150px; border: none; border-top: 1px solid black; margin-right: 10px;"/> <hr style="display: inline-block; width: 150px; border: none; border-top: 1px solid black; margin-right: 10px;"/> <hr style="display: inline-block; width: 150px; border: none; border-top: 1px solid black; margin-right: 10px;"/> <hr style="display: inline-block; width: 150px; border: none; border-top: 1px solid black; margin-right: 10px;"/>		
<u>PstI</u>	<u>SphI</u>	<u>HindIII</u>
<u>Pac I</u> (HindIII)		

In order to facilitate rapid and efficient purification by means of Ni²⁺ chelation of engineered histidine stretches fused to the recombinant proteins, the vaccinia recombination vector pMS66 was designed to express secreted proteins with an additional carboxy-terminal histidine tag. An oligonucleotide linker with SEQ ID NO 2/95, containing unique sites for 3 restriction enzymes generating blunt ends (Sma I, Stu I and Pml I/Bbr P1) was synthesized in such a way that the carboxy-terminal end of any cDNA could be inserted in frame with a sequence encoding the protease factor Xa cleavage site followed by a nucleotide sequence encoding 6 histidines and 2 stop codons (a new Pac I restriction site was also created downstream the 3'end). This oligonucleotide with SEQ ID NO 2/95 was introduced between the Xma I and Pst I sites of pgptATA18 (Figure 3).

Oligonucleotide linker with SEQ ID NO 2/95:

5'	CCGGG GAGGCC TGCACGTGATCGAGGGCAGACACCATCACCATCACTAATAGTTAATTAA CTGCA	3'
3'	C CTCCGGACGTGCACTAGCTCCGTCTGTGGTAGTGTTGGTAGTGATTATCAATTAAATT	G
<hr style="display: inline-block; width: 150px; border: none; border-top: 1px solid black; margin-right: 10px;"/> <hr style="display: inline-block; width: 150px; border: none; border-top: 1px solid black; margin-right: 10px;"/>		
<u>XmaI</u>		<u>PstI</u>

Plasmid pgptATA-18 contained within *Escherichia coli* MC1061(lambda) has been deposited under the terms of the Budapest Treaty at BCCM/LMBP (Belgium Coordinated Collections of microorganisms/Laboratorium voor Moleculaire Biologie – Plasmidencollectie, Universiteit Gent, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium), and bears accession number LMBP4486. Said deposit was made on January 9, 2002.

Example 2. Construction of HCV recombinant plasmids